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Cow's urine as a yellow gold for bioelectricity generation in low cost clayware microbial fuel cell

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ABSTRACT

Treatment of cow's urine was first time explored in clayware microbial fuel cell (MFC) by varying dilution to have different chemical oxygen demand (COD) in the feed. Improvement in power output of MFC was attained with increase in feed concentration from 1.5 to 3 kg COD/m^3; however further increase in influent COD up to 30 kg COD/m^3 decreased the power. Maximum power of 5.23 W/m^3 was attained in MFC fed with diluted urine of cow with COD concentration of 3 kg COD/m^3, which was seven-fold higher than MFC fed with raw urine. Nitrate removal of 77± 4.1% and carbohydrate removal of 80± 3.9% were achieved in MFC fed with 3 kg COD/m^3. Electrochemical analysis showed that electrogenic activity of anodic biofilm boosted at optimum feed concentration (3 kg COD/m^3) of cow's urine in anodic chamber. Using two MFCs, fed with diluted cow's urine, maximum voltage of 1.36 ± 0.05 V in series connection and maximum current of 48 A/m^3 in parallel connection were achieved. Thus, cow's urine can serve as sustainable yellow gold to harvest bioelectricity using low cost clayware MFC, and to curb the water pollution likely caused from cattle sheds.

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1. Introduction

Urine of cow is a good digestive, laxative, neutralizing agent against toxins with very high therapeutic index and it is good biofertilizer [1]. Also, cow's urine is a source of vitamins, minerals, ions such as Na, N, Mg, Fe, Si, Cl, Mg, P, K, S, citric, succinic acid, lactose, calcium salts, enzymes, carbolic acid, etc. [2]. Due to high energy content value, it can be considered as a yellow gold. However, the biggest problem associated with use of cow's urine for agricultural purpose is the reuse of anthropogenic and animal waste nutrients on arable land and social view for food consumption from such land. Amongst the different types of waste received to wastewater treatment plant, about 75% of the nitrogen present in domestic wastewater is originated from urine, which accounts only 1% of the total volume [3]. Another associated problem is the nitrogen loss through ammonia evaporation during spreading and storage, and it contains pathogens like *Salmonella typhimerium* and *Pseudomonas aeruginosa* [4]. Therefore, there is a need to focus on efficient treatment of such urine, generated in cattle sheds, to avoid possible harmful effects of urine such as hygienic risks, environmental pollution, and unpleasant smell.

Due to high concentration of nutrients, though cow's urine can be considered as a valuable resource for N, P and energy recovery, it is a pollutant if discharged untreated into the environment [5]. Most of the nitrogen in fresh cow's urine is available in the form of urea. Urine contains about 2% urea by weight, and each molecule of urea consists of four atoms of hydrogen, which are loosely bound to the molecule than the hydrogen atom in water. Splitting of these bonds would require less energy and generate electrons as well as protons more efficiently during chemical oxidation reactions. To harvest energy from these bonds in the form of direct electricity, bioelectrochemical technology such as microbial fuel cell (MFC) can offer a sustainable and effective solution simultaneously offering treatment to urine [6]. MFC works by employing live microbes, those feed on organic matter and nutrients present in urine for energy generation for their own metabolism, growth, and cell maintenance. Performance of MFC is governed by several factors such as operational conditions, substrate type, substrate concentration, type and source of microbial inoculums etc. [7,8]. Substrate concentration significantly affects power generation of the MFCs by controlling biological activity in anodic chamber [9].

Direct utilization of human urine for electricity generation in MFC has been reported [6] and the work was extended to charge

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Nomenclature

MFC	Microbial fuel cell
CPE	Constant phase element
COD	Chemical oxygen demand
USA	United State of America
DO	Dissolved oxygen
OCV	Open circuit voltage
OV	Operating voltage
SHE	Standard hydrogen electrode
Ag/AgCl	Silver/Silver Chloride reference electrode
CV	Cyclic voltammetry
C	Biocapacitance
ECSA	Electrochemical accessible surface area
EIS	Electrochemical impedance spectroscopy
NH_4^+-N	Ammonium nitrogen
AC	Alternating current
NO_3^--N	Nitrate nitrogen
TAN	Total ammonium nitrogen
i_0	Exchange current density
R_{ct}	Charge transfer resistance
β	Charge transfer coefficient
b	Tafel slope
Rp	Polarization resistance
Rs	Solution resistance
W	Warburg's diffusion element

mobile phone battery with stacked MFCs arrangement [10]. To utilize the energetic value of urine, researchers have used human urine [6], elephant's urine [11], cow's urine [4,12,13] as a substrate in MFC for bioelectricity generation. Maximum voltage generation of 1.1 V was reported in dual chamber MFC using cow dung and urine as fuel [13].

Power generation from cow's urine was studied only by couple of researchers by using salt-bridge MFC in which internal resistance was too high and attaining maximum urine treatment efficiency was not exploited [4,12,13]. Also, the fundamental electrochemical mechanisms have not been clearly understood for treatment of cow's urine in MFC. The aforementioned studies have not evaluated the effect of concentration of urine to enhance performance of the MFC. To overcome these shortfalls of earlier studies, the main objective of present study was to investigate the effect of different concentrations of cow's urine in the feed, measured as chemical oxygen demand (COD), on the performance of low cost clayware MFC with the help of electrochemical analyses. In order to scale-up the MFC technology, stacking of small scale MFC units is one of the option for harvesting the useful electricity while treating such waste. Thus, implementation of MFC implies (i) a plurality of units and (ii) grouping of the units into series and parallel electrical connections. To study the grouping effect of MFC units, effect of electrical connections of electrodes such as individual, series and parallel connections on the performance of MFC was also evaluated.

2. Material and methods

2.1. Urine collection

The urine of cow was collected from well maintained cow-shed located near to Kharagpur, India. Urine was collected from a single cow having age of about four years with average weight and fed with a uniform diet. The urine of cow was used fresh or within a week from collection having a pH of 5.8–6.3. Urine was stored at 4 °C and allowed 2–3 h to re-equilibrate to room temperature before it was fed in MFC.

2.2. MFC design and operation

The study was carried out in a dual chambered MFC with outer cathodic chamber volume of 2.5 L, made from plastic bucket and inner clayware pot as anodic chamber with working volume of 0.4 L (Fig. 1). The wall material of clayware pot itself served as a separator between anodic chamber and cathodic chamber. The anode and cathode were made up of carbon felt (Panex 35®, Zoltek Corporation, St. Louis, USA) with projected surface area of 394 cm^2 and 755 cm^2, respectively (Fig. S1).

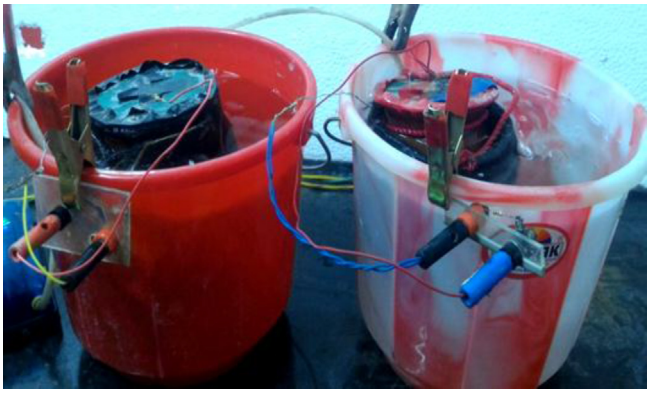

Fig. 1. Photograph of working dual chambered MFCs treating urine as a substrate in anodic chamber.

MFC was inoculated with mixed anaerobic sludge collected from septic tank bottom. The inoculum sludge was given heat pre-treatment at 100 °C for 15 min to suppress methanogens [14] and further cooled to room temperature before inoculation. The pre-treated sludge of 70 mL volume was added as inoculum in anodic chamber along with cow's urine, with different dilutions, as a substrate. To evaluate the effect of substrate concentration, MFC was fed with diluted cow's urine with COD concentrations of 1.5, 3, 3.75, 5, and 7.5 kg COD/m^3, and using raw urine of 30 kg COD/m^3 feed concentration in sequence under different batch cycles. The required COD concentration in the feed was attained by diluting the raw urine with tap water except at the feed concentration of 30 kg COD/m^3, where raw urine was used as feed. MFC was operated for 8 fed batch cycles of 5 days each under each feed COD concentration. Aerated tap water was used as catholyte and aeration was provided continuously with an aquarium pump (SOBO Aquarium air pump, China) to supply air at a constant rate of 3.5 mL/min.

Connections between the two electrodes were made with concealed copper wire through an external resistance of 100 Ω. For evaluating the effect of series and parallel connections of electrodes in stacked arrangement, the MFC mentioned above was fed with urine concentration of 3 kg COD/m^3 and another MFC with similar design was fabricated and fed with urine concentration of 5 kg COD/m^3. Initially these MFCs were electrically connected together in series and operated for 30 days and then connected in parallel for subsequent operation of 30 days. The performance of MFC was evaluated in terms of power generation and COD removal

efficiency during each cycle.

2.3. Water quality analyses

COD of anolyte soon after feeding (initial COD) and at the end of feed cycle (final COD) was analyzed by closed reflux colorimetric method as mentioned in Standard Methods [15]. Concentration of phenol was measured as per colorimetric method using 4-amino-antipyrine [15]. Total carbohydrate was estimated by Anthrone method [16] and protein was measured by Bradford method [17]. Concentration of ammonium-N in influent and effluent samples was analyzed as per standard methods [15]. Remaining water quality parameters such as dissolved oxygen (DO), nitrate (ion selective electrode), conductivity, resistivity, salinity and pH were measured using a water quality bench meter (Eutech Instruments, Cyber Scan, Singapore). The electrolyte temperature in MFC was measured regularly using thermometer.

2.4. Electrochemical analyses

2.4.1. Electrical parameters

Voltage was recorded using data acquisition unit (34972 A, Agilent Technologies, USA). The operating voltage was measured in parallel with the external resistance of 100 Ω, unless stated otherwise. Current generated by MFC was calculated by Ohm's law. Current density was calculated based on current generated from the MFC per unit surface area of anode under different operating conditions. Anodic and cathodic half-cell potentials were measured using Ag/AgCl reference electrode (+0.197 V vs. SHE, Bioanalytical Systems Inc., West Lafayette, Indiana, USA). Open circuit voltage (OCV) for each MFC was measured after allowing the circuit to remain in open circuit condition (no current flow) till the potential value stabilized [18].

2.4.2. Polarization behavior

The polarization and power density curves were plotted during stable power generation phase of MFC operation by varying external resistance from 20,000 Ω–1 Ω using resistance box (GEC05R Decade Resistance Box, Renown Systems, Kolkata, India) [19]. Volumetric power density (W/m^3), normalized to the effective volume of anolyte, was calculated by dividing the power with working volume of anodic chamber. The internal resistance of MFC was estimated using the maximum power point method from plot of power density curve [18]. Tafel plot analysis was used to understand the kinetics of electrode as per Eq. (1).

$$\eta = a + b\ ln\ i_o \tag{1}$$

where, i_o is exchange current density, η is overpotential, b is Tafel slope and a is an intercept of η vs. $ln\ i$ plot; i is the current density [20].

2.4.3. Cyclic voltammetry analysis

Cyclic voltammetry (CV) was used to confirm the current generation associated with biofilm formation during electrode reactions and oxidation–reduction processes [21] at different substrate concentrations in feed. In the present study, whole cell system was used for voltammetric analysis consisting of carbon felt anode as working electrode immersed in anolyte, Ag/AgCl (+0.197 V vs. SHE) as reference electrode, and cathode as counter electrode. A continuously time varying potential between working and reference electrode was controlled [22] with the help of potentiostat (Autolab PGSTAT 302 N, Utrecht, The Netherlands). The CV was performed at a scan rate of 10 mV/s by varying potential from −1.0 V to +1.0 V to measure the current response using NOVA 1.9 software. Bio-capacitance (C) was calculated following Eq. (2).

$$C = I \Big/ \left(A * \left(\frac{dV}{dt}\right)\right) \tag{2}$$

where, A is the electrode area, I is the peak current, and (dV/dt) is scan rate.

The electrochemical accessible surface area (ECSA) was estimated based on capacitance determined by cyclic voltammetry as per Eq. (3).

$$ECSA = \frac{(i_{ox} - i_{red})}{2 * \upsilon * C_{sp}} \tag{3}$$

where, i_{ox} and i_{red} are oxidation and reduction currents observed in CV analysis, respectively; v is scan rate; C_{sp} is specific capacitance of electrode material. The specific capacitance for the carbon felt is considered to be 35 $\mu F/cm^2$ [23]. The first derivative CV was calculated from turnover CV data by plotting the slope (dI/dE) of each CV data point against the applied potential (E).

2.4.4. Electrochemical impedance spectroscopy analysis

The electrochemical impedance spectroscopy (EIS) measurements (i.e. Nyquist plot and Bode plot analyses) were carried out for MFC in a frequency range of 100 kHz–1 mHz (with an AC signal of 10 mV amplitude) using Autolab PGSTAT 302 N potentiostat (The Netherlands) and NOVA 1.9 software. Polarization resistance, charge transfer resistance and diffusion resistance were calculated by using Nyquist plot and equivalent circuit in EIS [24]. All statistical tests for experimental data were carried out using Origin Pro 8 software (OriginLab Cooperation, MA, USA).

3. Results and discussion

3.1. Characterization of cow's urine

Influent raw urine had a high concentration of the organic matter (30 ± 0.28 kg COD/m^3) with high content of nitrogen and carbohydrate. It had a total carbohydrate of 1.980 ± 0.220 kg/m^3 with slightly acidic pH of 5.8–6.3. The average solution conductivity of fresh cow's urine was about 2.99 ± 0.23 S/m. Influent raw urine was pale yellow in colour and it contained about 0.109 ± 0.012 kg/m^3 of nitrate-N. Protein and phenol content of 0.281 ± 0.019 kg/m^3 and 0.807 ± 0.045 kg/m^3 were found in raw urine, respectively. High concentration of C, N and P make urine a pollutant source if it is discharged without any treatment into water bodies. The collected urine was turbid in appearance mainly due to the presence of phosphates and carbonates. The

Table 1
Characterization of raw cow's urine used in present study.

S. No.	Parameters	Unit	Value
1.	pH		5.8–6.3
2.	Conductivity	S/m	2.99 ± 0.23
3.	Salinity	psu[a]	19.45 ± 1.9
4.	Resistivity	Ω.m	0.334 ± 0.027
5.	Nitrate-N	kg/m^3	0.109 ± 0.012
6.	Temperature	°C	29 ± 4
7.	Carbohydrate	kg/m^3	1.980 ± 0.220
8.	Phenol	kg/m^3	0.80 ± 0.045
9.	Protein	kg/m^3	0.28 ± 0.019
10	COD	kg/m^3	30 ± 0.28
11.	NH_4^+-N	kg/m^3	0.68 ± 0.08
12.	Colour		Pale yellow

[a] psu = practical salinity unit.

characteristics of raw urine are presented in Table 1.

3.2. Treatment of urine in MFC at different COD concentrations

After 5 days retention time in anodic chamber of MFC under batch mode of operation, average COD removal efficiencies of 82 ± 4.1, 77 ± 3.7, 71 ± 3.8, 70 ± 2.9, 68 ± 3.1 and 45 ± 2.5% were attained in MFC fed with urine having COD concentrations of 1.5, 3, 3.75, 5, 7.5 and 30 kg COD/m^3, respectively (Table 2), during stable operation. Maximum COD removal of 82 ± 4.1% was attained in MFC fed with diluted urine of 1.5 kg COD/m^3, which decreased to 45 ± 2.5% for feed COD concentration of 30 kg COD/m^3 (Table 2). The COD removal was higher with lower COD concentration in the feed than that of raw urine. During oxidation process, total carbohydrate removal of about 80.0 ± 3.9% was obtained at feed concentration of 3 kg COD/m^3 in feed within 5 days of batch cycle. Similarly, the phenol removal was higher in MFC fed with diluted cow's urine (3 kg COD/m^3) than raw urine. Maximum phenol removal of 67.1 ± 2.6% was achieved in MFC with higher dilution of cow's urine (3 kg COD/m^3), probably due to the presence of *Pseudomonas aeruginosa* in urine [4]. Results showed that increase in COD load of cow's urine in feed from 3.75 to 30 kg COD/m^3 resulted into decrease in protein removal from 89 ± 4.4 to 57 ± 3.8%. The highest protein removal of 89 ± 4.4% was achieved in MFC fed with diluted urine concentration of 3.75 kg COD/m^3 (Table 2).

Nitrate removal of 79 ± 3.9, 77 ± 4.1, 73 ± 3.5, 71 ± 2.9, 70 ± 3.0 and 67 ± 3.4% was achieved in the MFC fed with COD concentration of 1.5, 3, 3.75, 5, 7.5 and 30 kg COD/m^3, respectively. Higher nitrate removal of 77 ± 4.1% was achieved at lower COD concentration of 3 kg COD/m^3 in the feed, which was considerably higher as compared to MFC fed with raw urine (67 ± 3.4%). At feed COD concentration of 1.5 kg COD/m^3, the resulting nitrate concentration in the feed was 0.061 ± 0.009 kg/m^3 and resulted into highest nitrate removal of 79 ± 3.9%. Decomposition of urea during treatment leads to an increase in concentration of ammonium-nitrogen and resulted in an increase in pH of effluent from 5.9 to 8.1, as reported earlier by Tice and Kim [25]. The ammonium-nitrogen removal of 56 ± 2.2% was achieved at 3 kg COD/m^3, which is comparatively higher than that for raw urine. Higher level of ammonium nitrogen (0.68 ± 0.08 kg/m^3) was present in raw urine and ammonium-nitrogen removal efficiency reduced to 45 ± 1.9% when raw urine was fed in MFC. During treatment colour of raw urine changed from pale yellow to light yellowish due to phenol removal along with other organic matter. Thus, the results represent that clayware MFC used in this experiment can be a suitable option for effective treatment of diluted cow's urine with appreciable nitrogen and carbohydrate removal.

3.3. Effect of concentration of cow's urine on electricity production in MFC

The substrate concentration determines the maximum amount of current or the charge which can be generated from the MFC in a particular time period [26]. Maximum OCV of 592 ± 17, 947 ± 41, 798 ± 36, 570 ± 27, 512 ± 21 and 420 ± 23 mV was achieved in MFC fed with urine concentration of 1.5, 3, 3.75, 5, 7.5 and 30 kg COD/m^3, respectively (Fig. 2 A). At an external resistance of 100 Ω, sustainable power density of 24.96 ± 0.49, 52.87 ± 4.50, 45.90 ± 3.26, 11.61 ± 0.96, 8.48 ± 0.72 and 6.32 ± 0.39 mW/m^2 was achieved for MFC fed with urine concentration of 1.5, 3, 3.75, 5, 7.5 and 30 kg COD/m^3, respectively. During forward polarization, maximum volumetric power density of 3.08, 5.23, 5.0, 1.14, 0.89 and 0.64 W/m^3 was obtained in MFC treating cow's urine with concentration of 1.5, 3, 3.75, 5, 7.5 and 30 kg COD/m^3, respectively, with corresponding internal resistance of 60, 50, 60, 100, 150 and 150 Ω (Fig. 2 B). The highest volumetric power output of 5.23 W/m^3 at lower internal resistance of 50 Ω was achieved for MFC when feed of 3 kg COD/m^3 was used. Power density observed in MFC at 3 kg COD/m^3 of feed concentration was significantly higher as compared to power production reported in previous studies for MFC treating animal urine such as elephant urine [11], raw cow's urine [12] and human urine [6]. Increase in energy harvested from MFC occured with increase in concentration of urine from 1.5 to 3 kg COD/m^3. However, further increase in COD concentration resulted into reduction in power density probably due to inhibition of enzymatic activity of bacteria by ammonium toxicity and intermediate product formation (i.e. nitrate, nitrite) at higher dose of nitrogen species, as stated earlier by Jadhav and Ghangrekar [27,28]. This decrease in electricity generation at higher organic loading over 3 kg COD/m^3 can be attributed to organic overloading. Organic overloading is considered as responsible for system imbalance, wherein accumulation of volatile fatty acids at higher substrate concentration decreases anolyte pH and results in ceasing of microbial activities of electrochemically active bacteria. Similar observations have been noted in 40 L capacity clayware MFC operated at organic loading rate more than 4.5 kg/m^3 day [29]. These results are in agreement with the findings of Nam et al. [9] where power density decreased by 21.6% when organic loading rate was increased from 1.92 to 4.80 kg COD/m^3 day.

At high substrate concentration, more molecules of substrate bind together to the active sites of the enzyme carrying electrons which results in inhibitory effect for enzyme and thus, reduces the electron transfer activity towards the anode [30]. At higher concentration of 30 kg COD/m^3, about 47.4 ± 3.2% of COD remained unutilized; which influenced bacterial metabolic activity and in turn affected the electron and proton generation process. Also at higher feed concentration of 5 kg COD/m^3, the proportion of ammonium that accumulates inside a cell increases and become dominant for further higher feed concentrations, which might have caused bacterial inhibition by altering intracellular pH conditions [27]. These results are in agreement with the findings of Nam et al. [27] in which effect of ammonium inhibition on exoelectrogenic bacteria in MFC was studied and reported that initial total ammonium nitrogen (TAN) concentrations more than 0.50 kg N/m^3 significantly lowered down the power generation in MFC. Thus, results of present experiment showed that the urine concentration

Table 2
Performance of MFC treating urine as a substrate at different COD concentrations.

Removal (%)	MFC fed with COD dose (kg COD/m^3)					
	1.5	3	3.75	5	7.5	30
COD removal	82 ± 4.1	77 ± 3.7	71 ± 3.8	70 ± 2.9	68 ± 3.1	45 ± 2.5
NO_3^--N removal	79 ± 3.9	77 ± 4.1	73 ± 3.5	71 ± 2.9	70 ± 3.0	67 ± 3.4
NH_4^+-N removal	61 ± 1.9	56 ± 2.2	52 ± 2.8	51 ± 1.6	47 ± 1.3	45 ± 1.9
Phenol removal	66 ± 2.3	67 ± 2.6	62 ± 2.9	56 ± 2.2	49 ± 2.1	37 ± 2.6
Carbohydrate removal	79 ± 4.2	80 ± 3.9	76 ± 2.9	75 ± 3.6	72 ± 3.2	70 ± 3.6
Protein removal	84 ± 4.7	82 ± 3.9	89 ± 4.4	84 ± 4.2	71 ± 4	57 ± 3.8

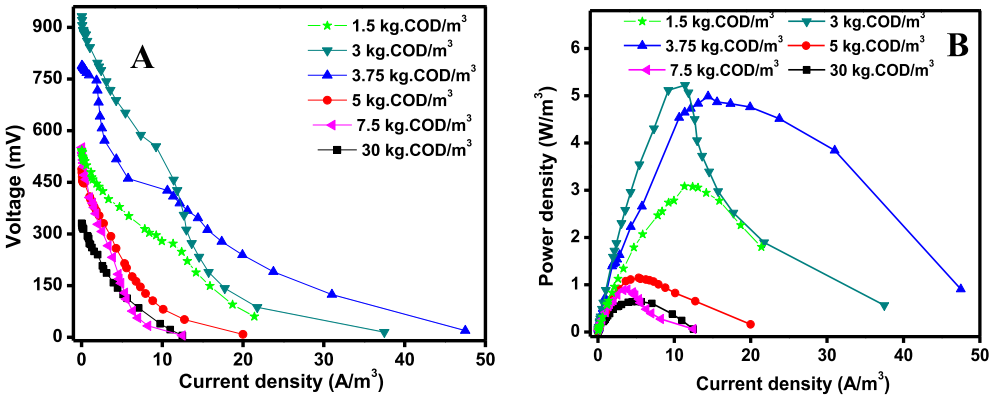

Fig. 2. (A) Polarization curve and (B) Power density curve of MFC operated at different feed substrate concentrations.

of 3 kg COD/m^3 is optimum for feeding in the MFC to obtain higher power output with higher COD removal.

3.4. Electrochemical analyses

3.4.1. Tafel analysis for evaluating kinetic activity of electrodes

Voltage drop due to activation losses in MFC can be expressed by a semi-empirical Tafel equation and can be plotted as logarithmic function of current density against overpoterntial of MFC under given operating conditions (Eq. (1)). Tafel analysis is a technique for evaluating the kinetic activity of electrodes that mainly correlates the exchange current density (i_0), charge transfer resistance (R_{ct}), charge transfer coefficient (β) and Tafel slope (b) with overpotential of electrodes [31]. The exchange current density (i_0) describes the current that the electrochemical cell is capable of producing without activation influence or other losses [20]. The i_0 value for anode of MFC increased from 0.503 to 1.18 mA/m^2 with increase in feed COD concentration from 1.5 to 3 kg COD/m^3; however the i_0 value decreased to 0.138 mA/m^2 with further increase in feed COD concentration to 30 kg COD/m^3 by supplying undiluted raw urine. Maximum i_0 of 1.18 and 0.65 mA/m^2 was obtained for anode and cathode, respectively, for MFC fed with 3 kg COD/m^3 of cow's urine (Table 3). Higher i_0 attained at 3 kg COD/m^3 feed concentration was mainly due to the lower overpotential at the anode, which indicates lower activation energy was required to overcome the voltage losses associated with MFC operated at higher external resistances [20].

The dimensionless parameter charge transfer coefficient (β) relates the properties of transition state for the forward and reverse reactions of reacting species in MFC. Lower β value ($0.29*10^{-3}$) in MFC fed with 3 kg COD/m^3 reflects the lower consumption of activation energy to bring the electron acceptor to the equilibrium state for faster electron transfer. Tafel analysis further revealed that the biofilm facilitated the electron transfer from the organic substrate molecule to the anode, increasing the oxidation rate of organic matter, and thus decreased the charge transfer resistance. Charge transfer resistance (R_{ct}) is inversely proportional to i_0 and related to the resistance offered to the flow of electrons and it is useful to deduce the kinetic rate constant of the redox processes that limits the electron transfer. R_{ct} increased from 21 Ω m^2 to 185 Ω m^2 with increase in feed COD concentration from 3 to 30 kg COD/m^3, respectively, indicating faster electrogenic activities in MFC treating cow's urine at COD concentration of 3 kg COD/m^3. Higher R_{ct} and lower i_0 resulted in reduction of performance of the MFC fed with raw urine, as the higher resistance offered to charge transfer reduced the electron transfer activity at higher concentration of substrate. This reduction in activity at higher substrate concentration might have been due to enzyme inhibitory kinetics because of higher concentration of ammonium [27] and decrease in electron transfer activity as discussed earlier in section 3.3. Also, high concentration of ammonium nitrogen in raw urine causes bacterial dehydration due to the altered osmotic pressure [32]. At higher concentration of urine (above 5 kg COD/m^3), electron transfer was decreased drastically due to oxidized nitrogen species present in substrate and possibly acting as electron acceptor.

Table 3
Tafel analysis for electrode in MFC treating cow's urine under different COD concentrations.

Substrate, kg COD/m^3	Electrode	i_0 mA/m^2	β x 10^3	R_{ct} Ω.m^2
1.5	Anode	0.503	0.84	51
	Cathode	0.487	0.04	52.6
3.0	Anode	1.18	0.29	21
	Cathode	0.65	0.34	39.26
3.75	Anode	0.96	0.11	26.7
	Cathode	0.64	6.69	39.8
5.0	Anode	0.75	0.08	34.3
	Cathode	0.42	0.05	61.23
7.5	Anode	0.467	0.248	54.9
	Cathode	0.219	9.49	88.1
30	Anode	0.138	0.8	185
	Cathode	0.251	0.21	102

3.4.2. Turnover cyclic voltammetry

Cyclic voltammetry (CV) is one of the important means to study electrochemical behavior of cell at different doses of substrate [33]. Under turnover conditions, an electron donor was provided to microbes, causing an electron flow with sufficient substrate available for redox reactions [24]. MFC fed with diluted cow's urine showed higher redox activity as compared to MFC treating raw urine. The oxidation current of 14, 41, 37, 33, 8 and 5 mA was achieved for MFC fed with COD concentration of 1.5, 3, 3.75, 5, 7.5 and 30 kg COD/m^3, respectively. With increase in feed concentration from 1.5 to 3 kg COD/m^3, three-fold increment in oxidative current (from 14 to 41 mA) was noted. The oxidative current was decreased from 41 to 5 mA with further increase in feed concentration from 3 to 30 kg COD/m^3. With lower COD concentration in the feed of 3.75 and 3 kg COD/m^3, the oxidative current was more prominent and redox current of about 37 and 41 mA, respectively, was observed (Fig. 3A).

The CV with substrate availability shows higher oxidative peaks in MFC fed with COD concentration of 3 kg COD/m^3. The decrease in electrochemical activities at higher concentration of urine was

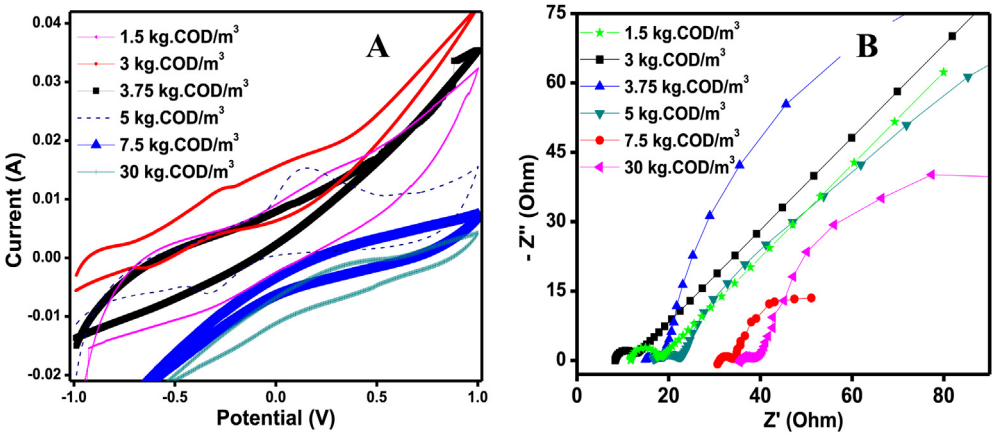

Fig. 3. (A) Cyclic voltammograms and (B) Nyquist plots for MFC fed with different urine concentrations.

mainly due to lower electron harvesting and charge holding in anodic chamber of MFC. The result implies that the affinity of electrogenic anodic microorganisms is greatly dependent on the availability of substrate at lower concentration, as stated by Nam et al. [9]. Voltammogram represents that the biofilm formed on the anode surface had a positive influence on current generation; and the rate of oxidation reactions were limited due to ammonium toxicity at high concentrations (over 7.5 kg COD/m^3). Higher ammonium concentration over 7.5 kg COD/m^3 results into disruption of enzymatic activity and further declines the rate of electron transfer from bacteria to the anode, as discussed earlier by Jadhav and Ghangrekar [28]. During turnover CV, less redox species were detected in MFC fed with raw urine and the redox current generated was low, thus decreasing the formal potential of MFC.

The first derivative of turnover CV revealed that the biofilm on anode of MFC fed with 3 kg COD/m^3 of urine exhibited more electro-activity over a wider range of anode potential, suggesting that electrogenic bacteria participated more in electron transfer at this substrate concentration and gained higher energy. The anodic bio-capacitance of 28, 103, 93, 84, 20 and 12 F/m^2 was obtained in MFC with urine concentration of 1.5, 3, 3.75, 5, 7.5 and 30 kg COD/m^3, respectively. The value of bio-capacitance of anode increased with increase in COD concentration upto 3 kg COD/m^3 and further decreased with increase in concentration, which represents the higher charge holding capacity of anode and better electro-catalytic activity at feed COD concentration of 3 kg COD/m^3. The increased charge harvesting capacity at this concentration correlates the increase in electrochemically active surface area (ECSA) of carbon felt electrode. Further increase in concentration from 3 to 5 kg COD/m^3 leads to decrease in ECSA from 176 to 96 cm^2. Thus, the power generation can be expressed as the function of ECSA and capacitance of electrode, which depends on the bio-electrochemical behavior of anodic biofilm at that particular substrate concentration [23].

3.4.3. Electrochemical impedance spectroscopy

EIS is a powerful tool for in-depth analysis of internal resistances in MFC. Generally the impedance spectra of MFC consist of polarization resistance (*Rp*), solution resistance (*Rs*) and Warburg's diffusion element (*W*) in parallel with a constant phase element (CPE) [34]. Nyquist plot during EIS analysis indicates that internal resistance of MFC can be expressed as a function of substrate concentration, which determines the performance of MFC. The semicircle at high frequency region is associated to electrical properties of anodic biofilm, and represented by *Rp*. With increase in feed COD concentration from 1.5 to 3 kg COD/m^3, *Rs* and *Rp* of cell decreased from 11.7 and 18.13 to 8.3 and 12.81 Ω, respectively; which increased further to 35.5 and 38.34 Ω, respectively, with increase in feed concentration from 3 to 30 kg COD/m^3 of cow's urine in MFC (Fig. 3B). The ohmic resistance of MFC treating cow's urine increased from 8.3 to 35.54 Ω with increase in COD concentration from 3 to 30 kg COD/m^3 (Table 4). However, charge transfer resistance (38.4 Ω) was more in MFC fed with raw urine than that of diluted urine. Also, Warburg resistance (*W*) increased as in case of MFC operated with raw urine mainly due to increase in diffusion and mass transport limitations [24]. The increase in diffusion resistance at higher loading was mainly due to the higher rate of substrate diffusion through the biofilm and the product of oxidation further diffused back to the anolyte containing urine as a substrate [35]. Increase value of constant phase element (CPE) represents enhanced charge holding capacity of MFC with lower organic load. Thus, the electrochemical analyses revealed that optimum COD concentration of 3 kg COD/m^3 of cow's urine was useful to achieve higher biocapacitance and to lower charge transfer resistance in MFC.

3.5. Performance of stacked MFC

To enhance the power output from MFC treating cow's urine, two MFCs were electrically connected together. First MFC received feed concentration of 3 kg COD/m^3 and second MFC received feed with concentration of 5 kg COD/m^3. When both MFCs were connected in series, the OCV of 1.36 ± 0.05 V (Fig. 4 A) was observed and sustainable power density of 53 mW/m^2 was obtained at 100 Ω external resistance. For repeatable 6 cycles, the series connection under open circuit condition resulted in 44% increase in voltage compared with the best performing individual MFC fed with 3 kg COD/m^3 (OCV of 0.947 V). During series connections, performance of MFC with 5 kg COD/m^3 was significantly lower than that fed with 3 kg COD/m^3; however, the OCV in series connection was addition of the individual OCV of both MFCs with no voltage loss. At an external resistance of 100 Ω, operating voltage of 0.456 V was observed while connecting two MFCs together in series; however numeric addition of voltage produced by each MFC did not occur due to other parasitic circuits involved and because of voltage reversal, as reported by previous researchers [36,37]. The phenomenon of voltage reversal occurred in series connection of two MFCs in which MFC with lower voltage output was charged by the MFC with higher voltage output. This confirms that MFC at 5 kg COD/m^3 limited the overall performance of the system when connected in series, as reported earlier by Wang et al. [38]. The reversal of polarization resulted due to poor biocatalytic response

Table 4
Electrical performance of MFC treating urine at different feed COD concentrations.

Parameter/MFC	MFC fed with COD dose (kg COD/m^3)						MFC connections	
	1.5	3	3.75	5	7.5	30	Series	Parallel
OCV, mV	592 ± 17	947 ± 41	798 ± 36	570 ± 27	512 ± 21	420 ± 23	1363 ± 49	962 ± 21
Operating voltage at 100 Ω resistance, mV	311 ± 13	449 ± 19	426 ± 22	219 ± 14	181 ± 16	152 ± 13	456 ± 28	421 ± 21
Power density[a], W/m^3	3.08	5.23	5	1.14	0.89	0.64	5.43	4.46
Current density[a], A/m^3	11.33	11.43	14.42	5.35	3.87	5.17	8.23	11.84
Internal resistance, Ω	60	50	60	100	150	150	90	185
Solution resistance, Ω	11.7	8.3	14.93	17	30.67	35.4	37.1	13.75
Polarization resistance, Ω	18.13	12.81	18.82	20.83	34.11	38.3	46.4	15.49

[a] Values obtained at an internal resistance by polarization method.

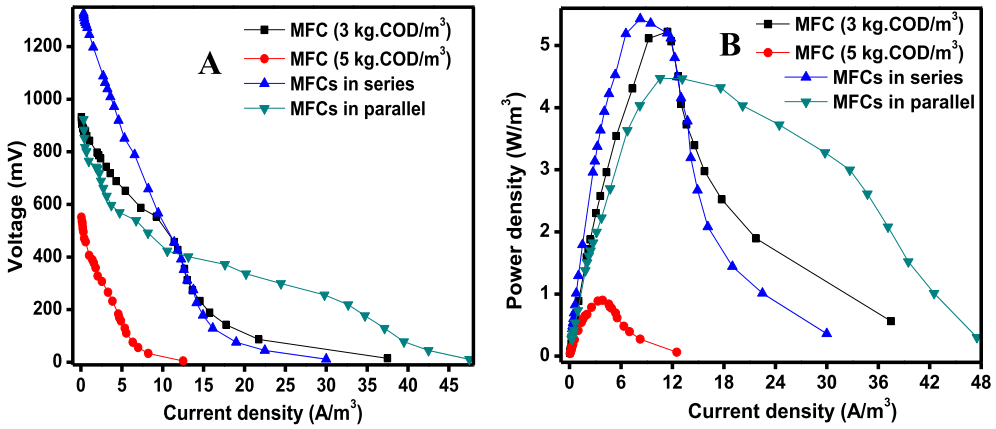

Fig. 4. (A) Polarization behavior and (B) Power density curve for MFCs with stacked connections.

of anodic biofilm at higher substrate concentration (5 kg COD/m^3) when two MFCs were connected in series.

Higher OCV of 1.36 ± 0.05 V was attained when two MFCs were connected in series; however, OCV of 0.962 V was achieved in parallel connection of MFCs. During parallel connection of both MFCs, maximum current reached upto 48 A/m^3 at lower external resistance of 1 Ω. During forward polarization, the maximum power produced from individual MFC fed with 3 and 5 kg COD/m^3 was 5.23 and 1.14 W/m^3, respectively. The volumetric power densities of 5.43 and 4.46 W/m^3 were obtained for series and parallel connection of both the MFCs, respectively (Fig. 4 B). The sustainable power density of 53 mW/m^2 obtained in present study was significantly higher than maximum power of 28 mW/m^2 obtained by Liu et al. [39] for two MFCs in series. While connecting MFCs in stack parallel electrode connection shall be preferred to integrate the current with use of DC-DC convertor for powering electronic appliances.

3.6. Urine degradation pathway

Urine is rich source of uric acid and urea. Uric acid ($C_5H_4N_4O_3$) is a hetero cyclic compound of C, H, and N which is useful for bacterial activities. During oxidation reaction at anode urate is produced [40]. In fresh urine, the major fraction of the nitrogen is present as urea which provides carbon and nitrogen source to bacteria. During oxidation of urea ($CO(NH_2)_2$) in anodic chamber of MFC, protons and electrons are released as an oxidation products and gases like CO_2 and N_2 are released as end products [41]. The overall reaction of urine treatment in MFC is shown below (Eq. (4) and Eq. (5)):

$$\text{Anode: } CO(NH_2)_2 + HOH \rightarrow N_2 + CO_2 + 6H^+ + 6e^- \quad (4)$$

$$\text{Cathode: } 1.5O_2 + 6H^+ + 6e^- \rightarrow 3H_2O \quad (5)$$

Overall reaction:

$$CO(NH_2)_2 + 1.5O_2 \rightarrow N_2 + 3H_2O + CO_2 + \text{electricity} \quad (6)$$

During oxidation, urease enzymes convert urea to CO_2 and NH_3. The bacteria use the nitrogen oxides for microbial respiration and the products result into increase in the pH of the anolyte. After hydrolysis, increase in pH was noted from 5.9 to 8.1, primarily due

Table 5
Comparing performance of MFC treating urine as a substrate with previous studies.

MFC	Size	MFC output	Details	Reference
Human urine	25 mL	8 mA/m^2	First study on urine utilization in MFC	[6]
Human urine	0.13 L	0.23 mA	Pt-based single chamber MFC	[44]
Human urine	6.25 mL	294 μW	struvite recovery & electricity in MFC	[45]
Elephant's urine	0.1 L	0.7 V	Elephant urine as substrate	[11]
Cow's urine	2 L	1.1 V	Cow dung and urine as substrate	[13]
Cow's urine	1.1 L	0.878 V (0.53 mA)	MFC with salt bridge	[4]
Cow's urine	1.5 L	0.8 V (with salt bridge)	MFC with and without salt bridge	[12]
Cow's urine	0.4 L	1.36 ± 0.05 V (series); 48 A/m^3 (parallel)	Effect of urine concentrations and stacked arrangement	Present study

to the formation of ammonium hydroxide. Finally N_2 gas is released as end-product of reaction (Eq. (6)). The electrons generated are used to harvest energy in the form electricity through electrical circuit. Thus, simultaneous urine degradation along with electricity generation was achieved by treating cow's urine in MFC.

3.7. Comparing performance of MFC with previous studies

Previously, studies have been carried out for generating power from human urine with use of power management system [6]. Recently, use of clayware membrane in MFC for treating human urine as a substrate was studied by Pasternak et al. [42] in small scale MFC. However, only limited study focused on use of cow's urine as a substrate and power generation from it (Table 5). Power generation observed in the present study, from low-cost clayware MFC fed with diluted urine (3 kg COD/m^3), was significantly higher as compared to previous studies focusing on cow's urine treatment in MFC [4,12]. The power output attained in MFC fed with optimum COD of 3 kg COD/m^3 was significantly higher as compared to MFC treating animal urine (Table 5) such as elephant urine [11], raw cow's urine [12], and even human urine [6]. The COD removal of 77 ± 3.7% attained at feed COD concentration of 3 kg COD/m^3 cow's urine was significantly higher than 65% reported in MFC treating human urine [43]. Effective treatment of cow's urine at different feed COD concentrations was first time explored in present study using low cost clayware MFC. The higher COD removal, low cost of fabrication and operation, inexpensive clayware separator, lower internal resistance are major features of MFC used in present study with determination of optimum feed COD concentration of urine. Results obtained in present study clearly indicate that urine of cow can serve as fuel (substrate) in MFC for generating bio-electricity.

4. Conclusion

Simultaneous urine treatment and electricity generation can be achieved in clayware MFC. Power generation from MFC fed with optimum concentration of urine (3 kg COD/m^3) was seven-fold higher than MFC treating raw urine. Electrochemical analyses revealed that biocatalytic activities in anodic chamber can be improved by feeding diluted urine in MFC upto optimum concentration of 3 kg COD/m^3. Maximum open circuit voltage of 1.36 ± 0.05 V and current of 48 A/m^3 were achieved in series and parallel connection of two MFCs, respectively. Thus, cow's urine can serve as sustainable yellow gold to harvest bioelectricity using low cost clayware MFC.

5. Future scope

In future, evaluating feasibility of simultaneous by-product recovery (such as Struvite, ammonia) and tertiary treatment to the anodic effluent in cathodic chamber of MFC will make MFC technology more effective and attractive to harvest maximum byproducts from urine.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.energy.2016.07.025.

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